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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: HARPOLD et al.

Serial No.:07/938,154

Filed: November 30, 1992

Group Art Unit: 1812

Examiner: Ulm, J.

For: HUMAN NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR COMPOSITIONS AND METHODS EMPLOYING SAME

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Edwin C. Johnson, hereby declare as follows:

1) I received a Ph.D. in Biology from Purdue University, West Lafayette, Indiana, in 1984. Following receipt of the Ph.D., I worked at Brandeis University, Waltham, Massachusetts, as a postdoctoral Fellow from 1984-1987; and then at Marshall University School of Medicine, Huntington, West Virginia, initially as an Assistant Professor, and later, as an Associate Professor, from 1987-1992.

2) I am currently a Senior Research Scientist at SIBIA Neurosciences Inc., La Jolla, California (formally THE SALK INSTITUTE BIOTECHNOLOGY/INDUSTRIAL ASSOCIATES INC., and hereinafter referred to as SIBIA). I have worked in the field of neurophysiology for at least 17 years. I am the author or co-author of more than 20 technical publications, primarily in the field of neuroscience.

3) In my capacity as a researcher at SIBIA, I have compared the functional properties of cloned nicotinic acetylcholine receptors (nAChR) from various species of animals. I have provided a DECLARATION executed January 27, 1995, demonstrating differences between rat and human nicotinic acetylcholine receptors.

4) A recent publication from SIBIA by Chavez-Noriega et al., entitled "Pharmacological Characterization of Recombinant Human Neuronal Nicotinic Acetylcholine Receptors hα2β2, hα2β4, hα3β2, hα3β4, hα4β2, hα4β4 and hα7 Expressed in Xenopus Oocytes" (J. Pharmacol. Exp. Ther., 280:346-356 (1997)), of which I am senior author, provides further evidence that human and rat nicotinic acetylcholine receptors (nAChR) have different pharmacological properties.

5) As described in Chavez-Noriega et al., recombinant human α and β nAChR subunits were expressed in xenopus oocytes to form active human nAChR and the oocytes were then evaluated for electrophysiological responses to various concentrations of nAChR agonists, including acetylcholine (AcCh), nicotine, cytisine (CYT) and 1,1-dimethyl-4-phenylpiperazinium (DMPP). The rank order potencies of the various agonists for the human nAChR was based on estimates of the EC_{50} from full dose-response curves. This was compared with literature values for rank order potency of recombinant rat nAChR that had been previously published by others. Comparison of the rank order of potency showed that human $\alpha 3\beta 2$, $\alpha 3\beta 4$ and $\alpha 7$ nAChRs were different from rat $\alpha 3\beta 2$, $\alpha 3\beta 4$ and $\alpha 7$ nAChRs(see e.g., Abstract and Discussion, page 353, 2nd Col. 2nd. full para.). Thus, these results further support the observation that the pharmacological properties of recombinant rat nAChR are not predictive of the cloned human nAChR. Full dose response curves for rat $\alpha 2\beta 2$ or $\alpha 2\beta 4$ nAChRs are not available in the literature, thereby precluding such comparison.

6) Figure 4 of Chavez-Noriega et al., shows partial dose response curves for each agonist with the particular human nAChR studied. The deduced rank order potency is based on a qualitative comparison of partial dose response curves for human $\alpha 2\beta 2$ and is stated to be similar to that published for the rat (see Discussion, page 353, 2nd Col. 2nd. full para.). This conclusion is not inconsistent with my prior statements regarding differences between these combinations of subunits and does not mean that rat and human $\alpha 2\beta 2$ are pharmacologically the same.

The prior DECLARATION provided data from a side-by-side comparison in which relatively low concentrations of the agonists were used. At other than low concnetrations of agonists, the receptors are subject to densitization and potential dampening of the response. In the experiments in the prior DECLARATION, $\alpha 2\beta 2$ and $\alpha 3\beta 2$ from human and rat were compared for rank order potency at the single low concentration of each agonist. These experiments demonstrated that $\alpha 2\beta 2$ of human and rat differed in rank order potency at the selected concentration.

To my knowledge, the comparison of pharmacological properties of recombinant human neuronal nicotinic acetylcholine receptors with those that of rat disclosed in my January 17, 1995 DECLARATION is the only quantitative side-by-side comparison performed in a manner that permits a meaningful comparison, whereby pharmacological differences could be observed.

In contrast, the experiments in Chavez-Noriega et al. with the human subunits were performed at SIBIA, but the data for the rat subunits was deduced from published reports. The comparisons from Chavez-Noriega et al. were based on a qualitative assessment of partial dose response curves.

Therefore, the results in the DECLARATION and the publication by Chavez-Noriega et al. demonstrate that functional properties of a human receptor cannot be predicted from the functional properties of a receptor from another species

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

5/12/97
Date

Edwin C. Johnson